



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/715, 16/28, A61K 38/00, G01N 33/00 // C07K 14/54	A1	(11) International Publication Number: WO 97/31946 (43) International Publication Date: 4 September 1997 (04.09.97)
(21) International Application Number: PCT/US97/03124 (22) International Filing Date: 28 February 1997 (28.02.97) (30) Priority Data: 08/609,572 1 March 1996 (01.03.96) US (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). (72) Inventors: COLLINS, Mary; 27 Euclid Avenue, Natick, MA 01760 (US). DONALDSON, Debra; 108 Blakely Street, Medford, MA 02155 (US). FITZ, Lori; 13 Palmer Street, Arlington, MA 02174 (US). NEBEN, Tamlyn; 13 Duggan Street, Acton, MA 01720 (US). WHITTERS, Matthew; 9 Vinal Street, Hudson, MA 01749 (US). WOOD, Clive; 2 Hawthorne Place #17R, Boston, MA 02114 (US). (74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140 (US).		(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: IL-13 BINDING CHAIN OF IL-13 RECEPTOR		
(57) Abstract Polynucleotides encoding the IL-13 receptor and fragments thereof are disclosed. IL-13 receptor proteins, methods for their production, inhibitors of binding of IL-13 and its receptor and methods for their identification are also disclosed.		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

IL-13 binding chain of IL-13 receptor

Field of the Invention

5 The present invention relates to mammalian cytokine receptor proteins with affinity for IL-13 (including without limitation human and murine receptor proteins), fragments thereof and recombinant polynucleotides and cells useful for expressing such proteins.

10 Background of the Invention

 A variety of regulatory molecules, known as cytokines, have been identified including interleukin-13 (IL-13). Various protein forms of IL-13 and DNA encoding various forms of IL-13 activity are described in McKenzie et al., Proc. Natl. Acad. Sci. USA 90:3735 (1993); Minty et al., Nature 362:248 (1993); and
15 Aversa et al., WO94/04680. Thus, the term "IL-13" includes proteins having the sequence and/or biological activity described in these documents, whether produced by recombinant genetic engineering techniques; purified from cell sources producing the factor naturally or upon induction with other factors; or synthesized by chemical techniques; or a combination of the foregoing.

20 IL-13 is a cytokine that has been implicated in production of several biological activities including: induction of IgG4 and IgE switching, including in human immature B cells (Punnonen et al., J. Immunol. 152:1094 (1994)); induction of germ line IgE heavy chain (ϵ) transcription and CD23 expression in normal human B cells (Punnonen et al., Proc. Natl. Acad. Sci. USA 90:3730 (1993)); and
25 induction of B cell proliferation in the presence of CD40L or anti-CD40 mAb (Cocks et al., Int. Immunol. 5:657 (1993)). Although many activities of IL-13 are similar to those of IL-4, in contrast to IL-4, IL-13 does not have growth promoting effects on activated T cells or T cell clones (Zurawski et al., EMBO J. 12:2663 (1993)).

 Like most cytokines, IL-13 exhibits certain biological activities by interacting with an IL-13 receptor ("IL-13R") on the surface of target cells. IL-

13R and the IL-4 receptor ("IL-4R") sharing a common component, which is required for receptor activation; however, IL-13 does not bind to cells transfected with the 130 kD IL-4R (Zurawski et al., *supra*). Thus, the IL-13R must contain at least one other ligand binding chain. Cytokine receptors are commonly composed or two or three chains. The cloning of one ligand binding chain for IL-13 has been recently reported (Hilton et al., Proc. Natl. Acad. Sci. 93:497-501).

It would be desirable to identify and clone the sequence for any other IL-13 binding chain of IL-13R so that IL-13R proteins can be produced for various reasons, including production of therapeutics and screening for inhibitors of IL-13 binding to the receptor and receptor signaling.

Summary of the Invention

In accordance with the present invention, polynucleotides encoding the IL-13 binding chains of the interleukin-13 receptor are disclosed, including without limitation those from the murine and human receptors. In certain embodiments, the invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 256 to nucleotide 1404;

(b) the nucleotide sequence of SEQ ID NO:3 from nucleotide 103 to nucleotide 1242;

(c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code;

(d) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a) or (b);

(e) a nucleotide sequence encoding a species homologue of the sequence specified in (a) or (b); and

(f) an allelic variant of the nucleotide sequence specified in (a) or (b).

Preferably, the nucleotide sequence encodes a protein having a biological activity of the human IL-13 receptor. The nucleotide sequence may be operably linked to an expression control sequence. In preferred embodiments, the polynucleotide

comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 256 to nucleotide 1404; the nucleotide sequence of SEQ ID NO:1 from nucleotide 319 to nucleotide 1257; the nucleotide sequence of SEQ ID NO:1 from nucleotide 1324 to nucleotide 1404; the nucleotide sequence of SEQ ID NO:3 from nucleotide 103 to nucleotide 1242; the nucleotide sequence of SEQ ID NO:3 from nucleotide 178 to nucleotide 1125; or the nucleotide sequence of SEQ ID NO:3 from nucleotide 1189 to nucleotide 1242.

The invention also provides isolated polynucleotides comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;
- (c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;
- (d) the amino acid sequence of SEQ ID NO:4;
- (e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;
- (f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and
- (g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain. Other preferred embodiments encode the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331 and the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

Host cells, preferably mammalian cells, transformed with the polynucleotides are also provided.

In other embodiments, the invention provides a process for producing a IL-13bc protein. The process comprises:

- (a) growing a culture of the host cell of the present invention in a suitable culture medium; and
- (b) purifying the human IL-13bc protein from the culture.

Proteins produced according to these methods are also provided.

The present invention also provides for an isolated IL-13bc protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- 5 (b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;
- (c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;
- (d) the amino acid sequence of SEQ ID NO:4;
- 10 (e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;
- (f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and
- (g) fragments of (a)-(f) having a biological activity of the IL-13
15 receptor binding chain

Preferably the protein comprises the amino acid sequence of SEQ ID NO:2; the sequence from amino acid 22 to 334 of SEQ ID NO:2; the sequence of SEQ ID NO:4; or the sequence from amino acid 26 to 341 of SEQ ID NO:4. In other preferred embodiments, the specified amino acid sequence is part of a fusion
20 protein (with an additional amino acid sequence not derived from IL-13bc). Preferred fusion proteins comprise an antibody fragment, such as an Fc fragment. Particularly preferred embodiments comprise the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331 and the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

25 Pharmaceutical compositions comprising a protein of the present invention and a pharmaceutically acceptable carrier are also provided.

The present invention further provides for compositions comprising an antibody which specifically reacts with a protein of the present invention.

30 Methods of identifying an inhibitor of IL-13 binding to the IL-13bc or IL-13 receptor are also provided. These methods comprise:

- (a) combining an IL-13bc protein or a fragment thereof with IL-13 or a fragment thereof, said combination forming a first binding mixture;
- (b) measuring the amount of binding between the protein and the IL-13 or fragment in the first binding mixture;
- 5 (c) combining a compound with the protein and the IL-13 or fragment to form a second binding mixture;
- (d) measuring the amount of binding in the second binding mixture; and
- (e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;
- 10 wherein the compound is capable of inhibiting IL-13 binding to the IL-13bc protein or IL-13 receptor when a decrease in the amount of binding of the second binding mixture occurs. Inhibitors of IL-13R identified by these methods and pharmaceutical compositions containing them are also provided.

15 Methods of inhibiting binding of IL-13 to the IL-13bc proteins or IL-13 receptor in a mammalian subject are also disclosed which comprise administering a therapeutically effective amount of a composition containing an IL-13bc protein, an IL-13bc or IL-13R inhibitor or an antibody to an IL-13bc protein.

20 Methods are also provided for potentiating IL-13 activity, which comprise combining a protein having IL-13 activity with a protein of claim 11 and contacting such combination with a cell expressing at least one chain of IL-13R other than IL-13bc. Preferably, the contacting step is performed by administering a therapeutically effective amount of such combination to a mammalian subject.

Brief Description of the Figure

25 The Figure presents photographs of IL-13, IL-4, IL-11 and mock transfected COS cells after exposure to IL-13bc-Fc as described in Example 4 below.

Detailed Description of Preferred Embodiments

30 The inventors of the present application have for the first time identified and provided polynucleotides encoding the IL-13 binding chain of IL-13R

(hereinafter "IL-13bc"), including without limitation polynucleotides encoding murine and human IL-13bc.

SEQ ID NO:1 provides the nucleotide sequence of a cDNA encoding the murine IL-13bc. SEQ ID NO:2 provides predicted the amino acid sequence of the receptor chain, including a putative signal sequence from amino acids 1-21. The mature murine IL-13bc is believed to have the sequence of amino acids 22-383 of SEQ ID NO:2. The mature murine receptor chain has at least three distinct domains: an extracellular domain (comprising approximately amino acids 22-334 of SEQ ID NO:2), a transmembrane domain (comprising approximately amino acids 335-356 of SEQ ID NO:2) and an intracellular domain (comprising approximately amino acids 357-383 of SEQ ID NO:2).

SEQ ID NO:3 provides the nucleotide sequence of a cDNA encoding the human IL-13bc. SEQ ID NO:4 provides predicted the amino acid sequence of the receptor chain, including a putative signal sequence from amino acids 1-25. The mature human IL-13bc is believed to have the sequence of amino acids 26-380 of SEQ ID NO:4. The mature human receptor chain has at least three distinct domains: an extracellular domain (comprising approximately amino acids 26-341 of SEQ ID NO:4), a transmembrane domain (comprising approximately amino acids 342-362 of SEQ ID NO:4) and an intracellular domain (comprising approximately amino acids 363-380 of SEQ ID NO:4).

The first 81 amino acids of the human IL-13bc sequence are identical to the translated sequence of an expressed sequence tag (EST) identified as "yg99f10.r1 Homo sapiens cDNA clone 41648 5'" and assigned database accession number R52795.gb_est2. There are no homologies or sequence motifs in this EST sequence which would lead those skilled in the art to identify the encoded protein as a cytokine receptor.

Soluble forms of IL-13bc protein can also be produced. Such soluble forms include without limitation proteins comprising amino acids 1-334 or 22-334 of SEQ ID NO:2 or amino acids 1-341 or 26-341 of SEQ ID NO:4. The soluble forms of the IL-13bc are further characterized by being soluble in aqueous solution, preferably at room temperature. IL-13bc proteins comprising only the intracellular

domain or a portion thereof may also be produced. Any forms of IL-13bc of less than full length are encompassed within the present invention and are referred to herein collectively with full length and mature forms as "IL-13bc" or "IL-13bc proteins." IL-13bc proteins of less than full length may be produced by expressing
5 a corresponding fragment of the polynucleotide encoding the full-length IL-13bc protein (SEQ ID NO:1 or SEQ ID NO:3). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis
10 methods or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "a biological activity of the IL-13 receptor binding chain" if it possess one or more of the following characteristics: (1) the ability to bind IL-13 or a fragment thereof
15 (preferably a biologically active fragment thereof); and/or (2) the ability to interact with the second non-IL-13-binding chain of IL-13R to produce a signal characteristic of the binding of IL-13 to IL-13R. Preferably, the biological activity possessed by the protein is the ability to bind IL-13 or a fragment hereof, more preferably with a K_D of about 0.1 to about 100 nM. Methods for determining
20 whether a particular protein or peptide has such activity include without limitation the methods described in the examples provided herein.

IL-13bc or active fragments thereof (IL-13bc proteins) may be fused to carrier molecules such as immunoglobulins. For example, soluble forms of the IL-13bc may be fused through "linker" sequences to the Fc portion of an
25 immunoglobulin. Other fusions proteins, such as those with GST, Lex-A or MBP, may also be used.

The invention also encompasses allelic variants of the nucleotide sequences as set forth in SEQ ID NO:1 or SEQ ID NO:3, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:1 or SEQ ID NO:3
30 which also encode IL-13bc proteins, preferably those proteins having a biological activity of IL-13bc. Also included in the invention are isolated polynucleotides

which hybridize to the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 under highly stringent conditions (for example, 0.1xSSC at 65°C). Isolated polynucleotides which encode IL-13bc proteins but which differ from the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 which are caused by point mutations or by induced modifications are also included in the invention.

The present invention also provides polynucleotides encoding homologues of the murine and human IL-13bc from other animal species, particularly other mammalian species. Species homologues can be identified and isolated by making probes or primers from the murine or human sequences disclosed herein and screening a library from an appropriate species, such as for example libraries constructed from PBMCs, thymus or testis of the relevant species.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the IL-13bc protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the IL-13bc protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the IL-13bc protein. Any cell type capable of expressing functional IL-13bc protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in

vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12, M1x or C2C12 cells.

5 The IL-13bc protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987),
10 incorporated herein by reference. Soluble forms of the IL-13bc protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the IL-13bc protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include
15 *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins.

Expression in bacteria may result in formation of inclusion bodies
20 incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active or more active material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve solubilizing the protein from the inclusion bodies, then denaturing the protein
25 completely using a chaotropic agent. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno, Meth. Enzym., 185:187-195 (1990). EP 0433225 and copending application USSN
30 08/163,877 describe other appropriate methods.

The IL-13bc protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the IL-13bc protein.

5 The IL-13bc protein of the invention may be prepared by growing a culture transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the IL-13bc protein of the invention can be purified from conditioned media. Membrane-bound forms of IL-13bc protein
10 of the invention can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

 The IL-13bc protein can be purified using methods known to those skilled in the art. For example, the IL-13bc protein of the invention can be concentrated
15 using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) or
20 polyethyleimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The
25 purification of the IL-13bc protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high
30 performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can

be employed to further purify the IL-13bc protein. Affinity columns including IL-13 or fragments thereof or including antibodies to the IL-13bc protein can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated IL-13bc protein is purified so that it is substantially free of other mammalian proteins.

IL-13bc proteins of the invention may also be used to screen for agents which are capable of binding to IL-13bc or IL-13R or which interfere with the binding of IL-13 to the IL-13 or IL-13bc (either the extracellular or intracellular domains) and thus may act as inhibitors of normal binding and cytokine action ("IL-13R inhibitors"). Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the IL-13bc protein of the invention. Purified cell based or protein based (cell free) screening assays may be used to identify such agents. For example, IL-13bc protein may be immobilized in purified form on a carrier and binding to purified IL-13bc protein may be measured in the presence and in the absence of potential inhibiting agents. A suitable binding assay may alternatively employ a soluble form of IL-13bc of the invention. Another example of a system in which inhibitors may be screened is described in Example 2 below.

In such a screening assay, a first binding mixture is formed by combining IL-13 or a fragment thereof and IL-13bc protein, and the amount of binding in the first binding mixture (B_0) is measured. A second binding mixture is also formed by combining IL-13 or a fragment thereof, IL-13bc protein, and the compound or agent to be screened, and the amount of binding in the second binding mixture (B) is measured. The amounts of binding in the first and second binding mixtures are compared, for example, by performing a calculation of the ratio B/B_0 . A compound or agent is considered to be capable of inhibiting binding if a decrease in binding in the second binding mixture as compared to the first binding mixture is observed. Optionally, the second chain of IL-13R can be added to one or both of the binding mixtures. The formulation and optimization of binding mixtures is

within the level of skill in the art. such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding. and additional control assays may be included in the screening assay of the invention.

5 Compounds found to reduce the binding activity of IL-13bc protein to IL-13 or its fragment to any degree, preferably by at least about 10%, more preferably greater than about 50% or more, may thus be identified and then secondarily screened in other binding assays and in vivo assays. By these means compounds having inhibitory activity for IL-13bc binding which may be suitable as therapeutic agents may be identified.

10 IL-13bc proteins, and polynucleotides encoding them, may also be used as diagnostic agents for detecting the expression or presence of IL-13bc, IL-13R, IL-13 or cells expressing IL-13bc, IL-13R or IL-13. The proteins or polynucleotides may be employed for such purpose in standard procedures for diagnostics assays using these types of materials. Suitable methods are well known to those skilled
15 in the art.

 IL-13bc acts as a mediator of the known biological activities of IL-13. As a result, IL-13bc protein (particularly, soluble IL-13bc proteins) and IL-13R inhibitors may be useful in treatment or modulation of various medical conditions in which IL-13 is implicated or which are effected by the activity (or lack thereof)
20 of IL-13 (collectively "IL-13-related conditions"). IL-13-related conditions include without limitation Ig-mediated conditions and diseases, particularly IgE-mediated conditions (including without limitation allergic conditions, asthma, immune complex diseases (such as, for example, lupus, nephrotic syndrome, nephritis, glomerulonephritis, thyroiditis and Grave's disease)); immune deficiencies,
25 specifically deficiencies in hematopoietic progenitor cells, or disorders relating thereto; cancer and other disease. Such pathological states may result from disease, exposure to radiation or drugs, and include, for example, leukopenia, bacterial and viral infections, anemia, B cell or T cell deficiencies such as immune cell or hematopoietic cell deficiency following a bone marrow transplantation. Since IL-
30 12 inhibits macrophage activation, IL-13bc proteins may also be useful to enhance

macrophage activation (i.e., in vaccination, treatment of mycobacterial or intracellular organisms, or parasitic infections).

IL-13bc proteins may also be used to potentiate the effects of IL-13 in vitro and in vivo. For example, an IL-13bc protein can be combined with a protein
5 having IL-13 activity (preferably IL-13) and the resulting combination can be contacted with a cell expressing at least one chain of IL-13R other than IL-13bc (preferably all chains of IL-13R other than IL-13bc). Preferably, the contacting step is performed by administering a therapeutically effective amount of such combination to a mammalian subject in vivo. The pre-established association of
10 the IL-13 protein with the IL-13bc protein will aid in formation of the complete IL-13/IL-13R complex necessary for proper signaling. See for example the methods described by Economides et al., Science 270:1351 (1995).

IL-13bc protein and IL-13R inhibitors, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a
15 pharmaceutically acceptable carrier. Such a composition may contain, in addition to IL-13bc or inhibitor and carrier, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The
20 characteristics of the carrier will depend on the route of administration.

The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15, G-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition
25 may also include anti-cytokine antibodies. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated IL-13bc
30 protein or IL-13bc inhibitor, or to minimize side effects caused by the isolated IL-13bc or IL-13bc inhibitor. Conversely, isolated IL-13bc or IL-13bc inhibitor may

be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

5 The pharmaceutical composition of the invention may be in the form of a liposome in which isolated IL-13bc protein or IL-13bc inhibitor is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for
10 liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are
15 incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of, healing of, or increase in rate of healing of such conditions. When
20 applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a
25 therapeutically effective amount of isolated IL-13bc protein or IL-13bc inhibitor is administered to a mammal. Isolated IL-13bc protein or IL-13bc inhibitor may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or
30 more cytokines, lymphokines or other hematopoietic factors, IL-13bc protein or IL-13bc inhibitor may be administered either simultaneously with the cytokine(s),

lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering IL-13bc protein or IL-13bc inhibitor in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of IL-13bc protein or IL-13bc inhibitor used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of IL-13bc protein or IL-13bc inhibitor is administered orally, IL-13bc protein or IL-13bc inhibitor will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% IL-13bc protein or IL-13bc inhibitor, and preferably from about 25 to 90% IL-13bc protein or IL-13bc inhibitor. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of IL-13bc protein or IL-13bc inhibitor, and preferably from about 1 to 50% IL-13bc protein or IL-13bc inhibitor.

When a therapeutically effective amount of IL-13bc protein or IL-13bc inhibitor is administered by intravenous, cutaneous or subcutaneous injection, IL-13bc protein or IL-13bc inhibitor will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous

cutaneous, or subcutaneous injection should contain, in addition to IL-13bc protein or IL-13bc inhibitor an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of IL-13bc protein or IL-13bc inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of IL-13bc protein or IL-13bc inhibitor with which to treat each individual patient. Initially, the attending physician will administer low doses of IL-13bc protein or IL-13bc inhibitor and observe the patient's response. Larger doses of IL-13bc protein or IL-13bc inhibitor may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 μ g to about 100 mg of IL-13bc protein or IL-13bc inhibitor per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the IL-13bc protein or IL-13bc inhibitor will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

IL-13bc proteins of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the IL-13bc protein and which may inhibit binding of IL-13 or fragments thereof to the receptor. Such antibodies may be obtained using the entire IL-13bc as an

immunogen, or by using fragments of IL-13bc, such as the soluble mature IL-13bc. Smaller fragments of the IL-13bc may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer. Chem. Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to IL-13bc protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies may be capable of blocking IL-13 binding to the IL-13bc.

Example 1

Isolation of IL-13bc cDNAs

Isolation of the murine IL-13 receptor chain.

5 ug of polyA⁺ RNA was prepared from the thymuses of 6-8 week old C3H/HeJ mice. Double stranded, hemimethylated cDNA was prepared using Stratagene's cDNA synthesis kit according to manufacturers instructions. Briefly, the first strand was primed with an oligodT-Xho primer, and after second strand synthesis, EcoRI adapters were added, and the cDNA was digested with XhoI, and purified. The cDNA was ligated to the XhoI-EcoRI sites of the Zap Express (Stratagene) lambda vector, and packaged using Gigapak II Gold packaging extracts (Stratagene) according to the manufacturers instructions. A library of 1.5×10^6 resulting recombinant phage was amplified following manufacturer's instructions. This library was screened with a degenerate 17mer oligonucleotide probe of the sequence KSRCTCCABKCRCTCCA (SEQ ID NO:5) (K = G+T; S = C+G; R = A+G; B = C+G+T) using standard TMAC hybridization conditions as described (Current Protocols in Molecular Biology, Ausubel, et al., editors., John Wiley and Sons, 1995, section 6.4.3). Clone A25 was identified because it hybridized to the 17mer probe, but not to probes derived from known

hematopoietin receptors. This clone was isolated in plasmid form from the ZapExpress vector as per manufacturers instruction. and the DNA sequence was determined. The DNA sequence encoded a novel member of the hematopoietin receptor family.

- 5 Clone A25 containing the polynucleotide having the sequence of SEQ ID NO:1 was deposited with ATCC as pA25pBKCMV at accession number 69997 on February 22, 1996.

Isolation of the human IL-13 receptor chain.

- 10 A partial fragment of the human homolog of the murine receptor was isolated by PCR using oligonucleotides derived from the murine sequence. cDNA was prepared from human testis polyA+ RNA that was obtained from Clontech. A DNA fragment of 274 base pairs was amplified from this cDNA by PCR with the following oligonucleotides: ATAGTTAAACCAT TGCCACC (SEQ ID NO:6) and CTCCATTCGCTCCAAATTCC (SEQ ID NO:7) using AmpliTaq polymerase (Promega) in 1X Taq buffer containing 1.5 mM MgCl₂ for 30 cycles of incubation (94°C x 1 minute, 42°C for 1 minute, and 72°C for 1 minute). The DNA sequence of this fragment was determined, and two oligonucleotides were prepared from an internal portion of this fragment with the following sequence:
- 15 AGTCTATCTTACTTTTACTCG (SEQ ID NO:8) and
20 CATCTGAGCAATAAATATTCAC (SEQ ID NO:9). These oligonucleotides were used as probes to screen a human testis cDNA library purchased from CLONTECH (cat #HL1161) . Filters were hybridized at 52°C using standard 5XSSC hybridization conditions and washed in 2X SSC at 52°C . Twenty two
25 clones were isolated that hybridized to both oligonucleotides in a screen of 400,000 clones. DNA sequence was determined from four of the cDNA clones, and all encoded the same novel hematopoietin receptor. The predicted DNA sequence of the full length human receptor chain is shown as SEQ ID NO:3.

- 30 The human clone was deposited with ATCC as phA25#11pDR2 at accession number 69998 on February 22, 1996.

Example 2Expression of Soluble IL-13bc Protein andAssay of Activity5 Production and purification of soluble IL-13bc-Ig.

DNA encoding amino acids 1-331 of the extracellular domain of murine IL-13bc was fused to a spacer sequence encoding gly-ser-gly by PCR and ligated in frame with sequences encoding the hinge CH2 CH3 regions of human IgG1 of the COS-1 expression vector pED.Fc. IL-13bc-Ig was produced from DEAE-dextran
10 transfected COS-1 cells and purified via protein A sepharose chromatography (Pharmacia).

B9 proliferation assay

Stimulation of proliferation of B9 cells (Aarden et al. Eur. J. Immunol.
15 1987. 17:1411-1416) in response to IL-13 or IL-4 was measured by 3H-thymidine incorporation into DNA. Cells (5 x 10³/well) were seeded into 96 well plates with media containing growth factors at varying concentrations in the presence or absence of IL-13bc-Ig at 1 µg/ml. After incubation for 3 days 1 µCi/well of 3H-thymidine was added and the cells incubated for an additional 4 hrs. Incorporated
20 radioactivity was determined using a LKB 1205 Plate reader.

The B9 cell line proliferated in response to IL-13, IL-4 or IL-6. Only responses to IL-13 were inhibited by the soluble IL-13bc-Ig, indicating that this receptor binds IL-13 specifically, but not IL-4 or IL-6. The tables show cpm. Two
25 separate experiments are shown.

cytokine dilution	IL-13 (3ng/ml)	IL-13 plus A25-Fc (1ug/ml)	IL-4 (20 ng/ml)	IL-4 plus A25-Fc (1 ug/ml)	Cos IL-6 (1/10,000)
1	37734	1943	6443	6945	37887
1/3	30398	1571	2680	2442	36500
1/10	16101	1461	1767	1771	33335
1/30	2148	1567	1619	1783	27271
1/100	1574	1419	1522	1576	18831
1/300	1512	1531	1373	1577	7768
1/1000	1316	1392	1190	1474	2760
1/3000	1834	1994	1482	1819	1672

5

10

20

15

cytokine dilution	IL-13 (3ng/ml)	IL-13 plus A25-Fc (5ug/ml)	IL-4 (20 ng/ml)	IL-4 plus A25-Fc (5ug/ml)	Cos IL-6 (1/10,000)	Cos IL-6 plus A25-Fc (5ug/ml)
1	6413	295	1216	1158	6969	7703
1/3	5432	281	518	656	7827	8804
1/10	2051	281	489	520	8345	10027
1/30	506	319	279	476	8680	9114
1/100	430	372	288	423	7426	10364
1/300	330	287	323	420	5531	6254
1/1000	326	389	348	nl	2524	nl
no cytokine	339	279	404	394	326	279

Example 3Direct Binding of Soluble IL-13bc to IL-13 Measured by Surface Plasmon
Resonance (Biacore Analysis).

5 A Biacore biosensor was used to measure directly the specific binding of
IL-13 to purified IL-13bc-Ig (Pharmacia. Johnsson et al., 1991). Approximately
10,000 to 17,000 resonance units (RU) of purified IL-13bc-Ig, human IgG1 or
irrelevant receptor were each covalently immobilized to different flow cells on the
sensor chip as recommended by the manufacturer. (RU's are a reflection of the
10 mass of protein bound to the sensor chip surface.) Purified IL-13 was injected
across the flow cells at 5 ul/min for 10 mins in the presence or absence of excess
purified IL-13bc-Ig. Binding was quantified as the difference in RU before and
after sample injection. Specific IL-13 binding of 481.9 RU was observed only for
immobilized IL-13bc-Ig whereas coinjection of IL-13 plus IL-13bc-Ig resulted in
15 no binding to the immobilized IL-13bc-Ig (4 RU). No IL-13 binding was observed
for either immobilized IgG or IL-11R-Ig (5.4 and 3.7 RU respectively).

Sample	IL-13BC-Ig (10,383 RU)	IgG control (13,399 RU)	IL-11R-Ig (17,182 RU)
100 ng/ml human IL-13	481.9 RU bound	5.4 RU bound	3.7 RU bound
100 ng/ml human IL-13 + soluble IL-13BC-Ig	4.0 RU bound	not tested	not tested

Example 4

Binding of IL-13 Expressed in COS Cells to

Labeled IL-13BC-Ig Fusion Protein:

COS in situ Detection of IL-13 with IL-13bc-Fc

5

Expression vectors for IL-13, IL-4, IL-11 or empty vector were transfected into COS-1 cells in duplicated plates via the DEAE-dextran method. Two days after transfection cells were washed twice in phosphate buffered saline (PBS) and fixed in the culture dish for 10' at 4° C with methanol. Following fixation cells were washed twice with PBS then rinsed once with binding buffer (PBS, 1% (w/v) bovine serum albumin, .1% (w/v) sodium azide) and incubated for two hours at 4° C in binding buffer with IL-13bc-Fc at 1.0ug/ml or with relevant anti-cytokine antisera. Cells were washed twice with PBS and incubated at 40 C with shaking in alkaline phosphatase labeled Rabbit F(ab)2' anti-human IgG diluted 1:500 in binding buffer (for Fc fusion detection) or Rabbit F(ab)2' anti-rat IgG (for anti-cytokine detection). Cells were again washed twice in PBS. Alkaline phosphatase activity was visualized using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate.

Specific binding was visualized under the microscope. Only cells transfected with IL-13 showed specific binding to IL13bc-Ig. (see photo of transfected cells, the Figure).

Example 5

Other Systems for Determination Biological Activity of IL-13bc Protein

Other systems can be used to determine whether a specific IL-13bc protein exhibits a "biological activity" of IL-13bc as defined herein. The following are examples of such systems.

Assays for IL-13 Binding

The ability of a IL-13bc protein to bind IL-13 or a fragment thereof can be determine by any suitable assays which can detect such binding. Some suitable examples follow.

5 Binding of IL-13 to the extracellular region of the IL-13bc protein will specifically cause a rapid induction of phosphotyrosine on the receptor protein. Assays for ligand binding activity as measured by induction of phosphorylation are described below.

10 Alternatively, a IL-13bc protein (such as, for example, a soluble form of the extracellular domain) is produced and used to detect IL-13 binding. For example, a DNA construct is prepared in which the extracellular domain (truncated prior, preferably immediately prior, to the predicted transmembrane domain) is ligated in frame to a cDNA encoding the hinge C_H2 and C_H3 domains of a human immunoglobulin (Ig) γ 1. This construct is generated in an appropriate expression vector for COS cells, such as pEDAC or pMT2. The plasmid is transiently
15 transfected into COS cells. The secreted IL-13bc-Ig fusion protein is collected in the conditioned medium and purified by protein A chromatography.

The purified IL-13bc-Ig fusion protein is used to demonstrate IL-13 binding in a number of applications. IL-13 can be coated onto the surface of an enzyme-linked immunosorbent assay (ELISA) plate, and then additional binding sites
20 blocked with bovine serum albumin or casein using standard ELISA buffers. The IL-13bc-Ig fusion protein is then bound to the solid-phase IL-13, and binding is detected with a secondary goat anti-human Ig conjugated to horseradish peroxidase. The activity of specifically bound enzyme can be measured with a colorimetric substrate, such as tetramethyl benzidine and absorbance readings.

25 IL-13 may also be expressed on the surface of cells, for example by providing a transmembrane domain or glucosyl phosphatidyl inositol (GPI) linkage. Cells expressing the membrane bound IL-13 can be identified using the IL-13bc-Ig fusion protein. The soluble IL-13bc-Ig fusion is bound to the surface of these cells and detected with goat anti-human Ig conjugated to a fluorochrome,
30 such as fluorescein isothiocyanate and flow cytometry.

Interaction Trap

A yeast genetic selection method, the "interaction trap" [Gyuris et al. Cell 75:791-803, 1993], can be used to determine whether a IL-13bc protein has a biological activity of IL-13bc as defined herein. In this system, the expression of reporter genes from both LexAop-Leu2 and LexAop-LacZ relies on the interaction between the bait protein, for example in this case a species which interacts with human IL-13bc, and the prey, for example in this case the human IL-13bc protein. Thus, one can measure the strength of the interaction by the level of Leu2 or LacZ expression. The most simple method is to measure the activity of the LacZ encoded protein, β -galactosidase. This activity can be judged by the degree of blueness on the X-Gal containing medium or filter. For the quantitative measurement of β -galactosidase activity, standard assays can be found in "Methods in Yeast Genetics" Cold Spring Harbor, New York, 1990 (by Rose, M.D., Winston, F., and Hieter, P.).

In such methods, if one wishes to determine whether the IL-13bc protein interacts with a particular species (such as, for example, a cytosolic protein which binds to the intracellular domain of the IL-13bc *in vivo*), that species can be used as the "bait" in the interaction trap with the IL-13bc protein to be tested serving as the "prey", or *vice versa*.

20

All patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Collins, Mary
Donaldson, Debra
Fitz, Lori
Neben, Tamlyn
Whitters, Matthew
Wood, Clive

(ii) TITLE OF INVENTION: CYTOKINE RECEPTOR CHAIN

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Genetics Institute, Inc.
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Brown, Scott A.
(B) REGISTRATION NUMBER: 32,724
(C) REFERENCE/DOCKET NUMBER: GI5268

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 498-8224
(B) TELEFAX: (617) 876-5851

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1525 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 256..1404

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCGGCA CGAGGGAGAG GAGGAGGGAA AGATAGAAAG AGAGAGAGAA AGATTGCTTG	60
CTACCCCTGA ACAGTGACCT CTCTCAAGAC AGTGCTTTGC TCTTCACGTA TAAGGAAGGA	120
AAACAGTAGA GATTCAATTT AGTGTCTAAT GTGGAAAGGA GGACAAAGAG GTCTTGTGAT	180
AACTGCCTGT GATAATACAT TTCTTGAGAA ACCATATTAT TGAGTAGAGC TTTCAGCACA	240
CTAAATCCTG GAGAA ATG GCT TTT GTG CAT ATC AGA TGC TTG TGT TTC ATT	291
Met Ala Phe Val His Ile Arg Cys Leu Cys Phe Ile	
1 5 10	
CTT CTT TGT ACA ATA ACT GGC TAT TCT TTG GAG ATA AAA GTT AAT CCT	339
Leu Leu Cys Thr Ile Thr Gly Tyr Ser Leu Glu Ile Lys Val Asn Pro	
15 20 25	
CCT CAG GAT TTT GAA ATA TTG GAT CCT GGA TTA CTT GGT TAT CTC TAT	387
Pro Gln Asp Phe Glu Ile Leu Asp Pro Gly Leu Leu Gly Tyr Leu Tyr	
30 35 40	
TTG CAA TGG AAA CCT CCT GTG GTT ATA GAA AAA TTT AAG GGC TGT ACA	435
Leu Gln Trp Lys Pro Pro Val Val Ile Glu Lys Phe Lys Gly Cys Thr	
45 50 55 60	
CTA GAA TAT GAG TTA AAA TAC CGA AAT GTT GAT AGC GAC AGC TGG AAG	483
Leu Glu Tyr Glu Leu Lys Tyr Arg Asn Val Asp Ser Asp Ser Trp Lys	
65 70 75	
ACT ATA ATT ACT AGG AAT CTA ATT TAC AAG GAT GGG TTT GAT CTT AAT	531
Thr Ile Ile Thr Arg Asn Leu Ile Tyr Lys Asp Gly Phe Asp Leu Asn	
80 85 90	
AAA GGC ATT GAA GGA AAG ATA CGT ACG CAT TTG TCA GAG CAT TGT ACA	579
Lys Gly Ile Glu Gly Lys Ile Arg Thr His Leu Ser Glu His Cys Thr	
95 100 105	
AAT GGA TCA GAA GTA CAA AGT CCA TGG ATA GAA GCT TCT TAT GGG ATA	627
Asn Gly Ser Glu Val Gln Ser Pro Trp Ile Glu Ala Ser Tyr Gly Ile	
110 115 120	
TCA GAT GAA GGA AGT TTG GAA ACT AAA ATT CAG GAC ATG AAG TGT ATA	675
Ser Asp Glu Gly Ser Leu Glu Thr Lys Ile Gln Asp Met Lys Cys Ile	
125 130 135 140	
TAT TAT AAC TGG CAG TAT TTG GTC TGC TCT TGG AAA CCT GGC AAG ACA	723

Tyr Tyr Asn Trp Gln Tyr Leu Val Cys Ser Trp Lys Pro Gly Lys Thr	
145 150 155	
GTA TAT TCT GAT ACC AAC TAT ACC ATG TTT TTC TGG TAT GAG GGC TTG	771
Val Tyr Ser Asp Thr Asn Tyr Thr Met Phe Phe Trp Tyr Glu Gly Leu	
160 165 170	
GAT CAT GCC TTA CAG TGT GCT GAT TAC CTC CAG CAT GAT GAA AAA AAT	819
Asp His Ala Leu Gln Cys Ala Asp Tyr Leu Gln His Asp Glu Lys Asn	
175 180 185	
GTT GGA TGC AAA CTG TCC AAC TTG GAC TCA TCA GAC TAT AAA GAT TTT	867
Val Gly Cys Lys Leu Ser Asn Leu Asp Ser Ser Asp Tyr Lys Asp Phe	
190 195 200	
TTT ATC TGT GTT AAT GGA TCT TCA AAG TTG GAA CCC ATC AGA TCC AGC	915
Phe Ile Cys Val Asn Gly Ser Ser Lys Leu Glu Pro Ile Arg Ser Ser	
205 210 215 220	
TAT ACA GTT TTT CAA CTT CAA AAT ATA GTT AAA CCA TTG CCA CCA GAA	963
Tyr Thr Val Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro Pro Glu	
225 230 235	
TTC CTT CAT ATT AGT GTG GAG AAT TCC ATT GAT ATT AGA ATG AAA TGG	1011
Phe Leu His Ile Ser Val Glu Asn Ser Ile Asp Ile Arg Met Lys Trp	
240 245 250	
AGC ACA CCT GGA GGA CCC ATT CCA CCA AGG TGT TAC ACT TAT GAA ATT	1059
Ser Thr Pro Gly Gly Pro Ile Pro Pro Arg Cys Tyr Thr Tyr Glu Ile	
255 260 265	
GTG ATC CGA GAA GAC GAT ATT TCC TGG GAG TCT GCC ACA GAC AAA AAC	1107
Val Ile Arg Glu Asp Asp Ile Ser Trp Glu Ser Ala Thr Asp Lys Asn	
270 275 280	
GAT ATG AAG TTG AAG AGG AGA GCA AAT GAA AGT GAA GAC CTA TGC TTT	1155
Asp Met Lys Leu Lys Arg Arg Ala Asn Glu Ser Glu Asp Leu Cys Phe	
285 290 295 300	
TTT GTA AGA TGT AAG GTC AAT ATA TAT TGT GCA GAT GAT GGA ATT TGG	1203
Phe Val Arg Cys Lys Val Asn Ile Tyr Cys Ala Asp Asp Gly Ile Trp	
305 310 315	
AGC GAA TGG AGT GAA GAG GAA TGT TGG GAA GGT TAC ACA GGG CCA GAC	1251
Ser Glu Trp Ser Glu Glu Glu Cys Trp Glu Gly Tyr Thr Gly Pro Asp	
320 325 330	
TCA AAG ATT ATT TTC ATA GTA CCA GTT TGT CTT TTC TTT ATA TTC CTT	1299
Ser Lys Ile Ile Phe Ile Val Pro Val Cys Leu Phe Phe Ile Phe Leu	
335 340 345	
TTG TTA CTT CTT TGC CTT ATT GTG GAG AAG GAA GAA CCT GAA CCC ACA	1347
Leu Leu Leu Cys Leu Ile Val Glu Lys Glu Glu Pro Glu Pro Thr	
350 355 360	

TTG AGC CTC CAT GTG GAT CTG AAC AAA GAA GTG TGT GCT TAT GAA GAT 1395
 Leu Ser Leu His Val Asp Leu Asn Lys Glu Val Cys Ala Tyr Glu Asp
 365 370 375 380

ACC CTC TGT TAAACCACCA ATTCTTGAC ATAGAGCCAG CCAGCAGGAG 1444
 Thr Leu Cys

TCATATTAAA CTCAATTTCT CTTAAATTT CGAATACATC TTCTTGAAAA TCCAAAAAAA 1504
 AAAAAAAAAA AAAAAGCTCGA G 1525

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 383 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Phe Val His Ile Arg Cys Leu Cys Phe Ile Leu Leu Cys Thr
 1 5 10 15
 Ile Thr Gly Tyr Ser Leu Glu Ile Lys Val Asn Pro Pro Gln Asp Phe
 20 25 30
 Glu Ile Leu Asp Pro Gly Leu Leu Gly Tyr Leu Tyr Leu Gln Trp Lys
 35 40 45
 Pro Pro Val Val Ile Glu Lys Phe Lys Gly Cys Thr Leu Glu Tyr Glu
 50 55 60
 Leu Lys Tyr Arg Asn Val Asp Ser Asp Ser Trp Lys Thr Ile Ile Thr
 65 70 75 80
 Arg Asn Leu Ile Tyr Lys Asp Gly Phe Asp Leu Asn Lys Gly Ile Glu
 85 90 95
 Gly Lys Ile Arg Thr His Leu Ser Glu His Cys Thr Asn Gly Ser Glu
 100 105 110
 Val Gln Ser Pro Trp Ile Glu Ala Ser Tyr Gly Ile Ser Asp Glu Gly
 115 120 125
 Ser Leu Glu Thr Lys Ile Gln Asp Met Lys Cys Ile Tyr Tyr Asn Trp
 130 135 140
 Gln Tyr Leu Val Cys Ser Trp Lys Pro Gly Lys Thr Val Tyr Ser Asp
 145 150 155 160
 Thr Asn Tyr Thr Met Phe Phe Trp Tyr Glu Gly Leu Asp His Ala Leu

165	170	175
Gln Cys Ala Asp Tyr Leu Gln His Asp Glu Lys Asn Val Gly Cys Lys		
180	185	190
Leu Ser Asn Leu Asp Ser Ser Asp Tyr Lys Asp Phe Phe Ile Cys Val		
195	200	205
Asn Gly Ser Ser Lys Leu Glu Pro Ile Arg Ser Ser Tyr Thr Val Phe		
210	215	220
Gln Leu Gln Asn Ile Val Lys Pro Leu Pro Pro Glu Phe Leu His Ile		
225	230	235
Ser Val Glu Asn Ser Ile Asp Ile Arg Met Lys Trp Ser Thr Pro Gly		
245	250	255
Gly Pro Ile Pro Pro Arg Cys Tyr Thr Tyr Glu Ile Val Ile Arg Glu		
260	265	270
Asp Asp Ile Ser Trp Glu Ser Ala Thr Asp Lys Asn Asp Met Lys Leu		
275	280	285
Lys Arg Arg Ala Asn Glu Ser Glu Asp Leu Cys Phe Phe Val Arg Cys		
290	295	300
Lys Val Asn Ile Tyr Cys Ala Asp Asp Gly Ile Trp Ser Glu Trp Ser		
305	310	315
Glu Glu Glu Cys Trp Glu Gly Tyr Thr Gly Pro Asp Ser Lys Ile Ile		
325	330	335
Phe Ile Val Pro Val Cys Leu Phe Phe Ile Phe Leu Leu Leu Leu Leu		
340	345	350
Cys Leu Ile Val Glu Lys Glu Glu Pro Glu Pro Thr Leu Ser Leu His		
355	360	365
Val Asp Leu Asn Lys Glu Val Cys Ala Tyr Glu Asp Thr Leu Cys		
370	375	380

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1369 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 103..1245

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGATCCGCGC GGATGAAGGC TATTTGAAGT CGCCATAACC TGGTCAGAAG TGTGCCTGTC	60
GGCGGGGAGA GAGGCAATAT CAAGGTTTTA AATCTCGGAG AA ATG GCT TTC GTT	114
Met Ala Phe Val	
1	
TGC TTG GCT ATC GGA TGC TTA TAT ACC TTT CTG ATA AGC ACA ACA TTT	162
Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile Ser Thr Thr Phe	
5 10 15 20	
GGC TGT ACT TCA TCT TCA GAC ACC GAG ATA AAA GTT AAC CCT CCT CAG	210
Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val Asn Pro Pro Gln	
25 30 35	
GAT TTT GAG ATA GTG GAT CCC GGA TAC TTA GGT TAT CTC TAT TTG CAA	258
Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr Leu Tyr Leu Gln	
40 45 50	
TGG CAA CCC CCA CTG TCT CTG GAT CAT TTT AAG GAA TGC ACA GTG GAA	306
Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu Cys Thr Val Glu	
55 60 65	
TAT GAA CTA AAA TAC CGA AAC ATT GGT AGT GAA ACA TGG AAG ACC ATC	354
Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr Trp Lys Thr Ile	
70 75 80	
ATT ACT AAG AAT CTA CAT TAC AAA GAT GGG TTT GAT CTT AAC AAG GGC	402
Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp Leu Asn Lys Gly	
85 90 95 100	
ATT GAA GCG AAG ATA CAC ACG CTT TTA CCA TGG CAA TGC ACA AAT GGA	450
Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln Cys Thr Asn Gly	
105 110 115	
TCA GAA GTT CAA AGT TCC TGG GCA GAA ACT ACT TAT TGG ATA TCA CCA	498
Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr Trp Ile Ser Pro	
120 125 130	
CAA GGA ATT CCA GAA ACT AAA GTT CAG GAT ATG GAT TGC GTA TAT TAC	546
Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp Cys Val Tyr Tyr	
135 140 145	
AAT TGG CAA TAT TTA CTC TGT TCT TGG AAA CCT GGC ATA GGT GTA CTT	594
Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly Ile Gly Val Leu	
150 155 160	
CTT GAT ACC AAT TAC AAC TTG TTT TAC TGG TAT GAG GGC TTG GAT CAT	642
Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu Gly Leu Asp His	
165 170 175 180	

GCA TTA CAG TGT GTT GAT TAC ATC AAG GCT GAT GGA CAA AAT ATA GGA Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly Gln Asn Ile Gly 185 190 195	690
TGC AGA TTT CCC TAT TTG GAG GCA TCA GAC TAT AAA GAT TTC TAT ATT Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys Asp Phe Tyr Ile 200 205 210	738
TGT GTT AAT GGA TCA TCA GAG AAC AAG CCT ATC AGA TCC AGT TAT TTC Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg Ser Ser Tyr Phe 215 220 225	786
ACT TTT CAG CTT CAA AAT ATA GTT AAA CCT TTG CCG CCA GTC TAT CTT Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro Pro Val Tyr Leu 230 235 240	834
ACT TTT ACT CGG GAG AGT TCA TGT GAA ATT AAG CTG AAA TGG AGC ATA Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu Lys Trp Ser Ile 245 250 255 260	882
CCT TTG GGA CCT ATT CCA GCA AGG TGT TTT GAT TAT GAA ATT GAG ATC Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr Glu Ile Glu Ile 265 270 275	930
AGA GAA GAT GAT ACT ACC TTG GTG ACT GCT ACA GTT GAA AAT GAA ACA Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val Glu Asn Glu Thr 280 285 290	978
TAC ACC TTG AAA ACA ACA AAT GAA ACC CGA CAA TTA TGC TTT GTA GTA Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu Cys Phe Val Val 295 300 305	1026
AGA AGC AAA GTG AAT ATT TAT TGC TCA GAT GAC GGA ATT TGG AGT GAG Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly Ile Trp Ser Glu 310 315 320	1074
TGG AGT GAT AAA CAA TGC TGG GAA GGT GAA GAC CTA TCG AAG AAA ACT Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu Ser Lys Lys Thr 325 330 335 340	1122
TTG CTA CGT TTC TGG CTA CCA TTT GGT TTC ATC TTA ATA TTA GTT ATA Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu Ile Leu Val Ile 345 350 355	1170
TTT GTA ACC GGT CTG CTT TTG CGT AAG CCA AAC ACC TAC CCA AAA ATG Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr Tyr Pro Lys Met 360 365 370	1218
ATT CCA GAA TTT TTC TGT GAT ACA TGA AGACTTTCCA TATCAAGAGA Ile Pro Glu Phe Phe Cys Asp Thr + 375 380	1265
CATGGTATTG ACTCAACAGT TTCCAGTCAT GGCCAAATGT TCAATATGAG TCTCAATAAA CTGAATTTTT CTTGCGAAAA AAAAAAAAAA AAATCCGCGG ATCC	1325 1369

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 380 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Met Ala Phe Val Cys Leu Ala Ile Gly Cys Leu Tyr Thr Phe Leu Ile
 1             5             10             15

Ser Thr Thr Phe Gly Cys Thr Ser Ser Ser Asp Thr Glu Ile Lys Val
      20             25             30

Asn Pro Pro Gln Asp Phe Glu Ile Val Asp Pro Gly Tyr Leu Gly Tyr
      35             40             45

Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
      50             55             60

Cys Thr Val Glu Tyr Glu Leu Lys Tyr Arg Asn Ile Gly Ser Glu Thr
      65             70             75             80

Trp Lys Thr Ile Ile Thr Lys Asn Leu His Tyr Lys Asp Gly Phe Asp
      85             90             95

Leu Asn Lys Gly Ile Glu Ala Lys Ile His Thr Leu Leu Pro Trp Gln
      100            105            110

Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
      115            120            125

Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
      130            135            140

Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
      145            150            155            160

Ile Gly Val Leu Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu
      165            170            175

Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
      180            185            190

Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
      195            200            205

Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
      210            215            220

Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro

```

225					230					235					240
Pro	Val	Tyr	Leu	Thr	Phe	Thr	Arg	Glu	Ser	Ser	Cys	Glu	Ile	Lys	Leu
				245					250					255	
Lys	Trp	Ser	Ile	Pro	Leu	Gly	Pro	Ile	Pro	Ala	Arg	Cys	Phe	Asp	Tyr
			260					265					270		
Glu	Ile	Glu	Ile	Arg	Glu	Asp	Asp	Thr	Thr	Leu	Val	Thr	Ala	Thr	Val
		275					280					285			
Glu	Asn	Glu	Thr	Tyr	Thr	Leu	Lys	Thr	Thr	Asn	Glu	Thr	Arg	Gln	Leu
	290					295					300				
Cys	Phe	Val	Val	Arg	Ser	Lys	Val	Asn	Ile	Tyr	Cys	Ser	Asp	Asp	Gly
305					310					315					320
Ile	Trp	Ser	Glu	Trp	Ser	Asp	Lys	Gln	Cys	Trp	Glu	Gly	Glu	Asp	Leu
				325					330				335		
Ser	Lys	Lys	Thr	Leu	Leu	Arg	Phe	Trp	Leu	Pro	Phe	Gly	Phe	Ile	Leu
			340					345					350		
Ile	Leu	Val	Ile	Phe	Val	Thr	Gly	Leu	Leu	Leu	Arg	Lys	Pro	Asn	Thr
		355					360					365			
Tyr	Pro	Lys	Met	Ile	Pro	Glu	Phe	Phe	Cys	Asp	Thr	*			
	370					375						380			

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

KSRICTCCABK CRCTCCA

17

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAGTTAAAC CATTGCCACC

20

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCCATTTCG TCCTAAATTC

20

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGTCTATCTT ACTTTTACTC G

21

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATCTGAGCA ATAAATATTC AC

22

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
 - (a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 256 to nucleotide 1404;
 - (b) the nucleotide sequence of SEQ ID NO:3 from nucleotide 103 to nucleotide 1242;
 - (c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code;
 - (d) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a) or (b);
 - (e) a nucleotide sequence encoding a species homologue of the sequence specified in (a) or (b); and
 - (f) an allelic variant of the nucleotide sequence specified in (a) or (b).
2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes for a protein having a biological activity of the IL-13R binding chain.
3. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.
4. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 319 to nucleotide 1257.

5. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 1324 to nucleotide 1404.
6. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 178 to nucleotide 1125.
7. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 1189 to nucleotide 1242.
8. A host cell transformed with the polynucleotide of claim 3.
9. The host cell of claim 8, wherein said cell is a mammalian cell.
10. A process for producing a IL-13bc protein, said process comprising:
 - (a) growing a culture of the host cell of claim 8 in a suitable culture medium;and
 - (b) purifying the IL-13bc protein from the culture.
11. An isolated IL-13bc protein comprising an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:2;
 - (b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;
 - (c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;

- (d) the amino acid sequence of SEQ ID NO:4;
 - (e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;
 - (f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and
 - (g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain.
12. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:2.
13. The protein of claim 11 comprising the sequence from amino acid 22 to 334 of SEQ ID NO:2.
14. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:4.
15. The protein of claim 11 comprising the sequence from amino acid 26 to 341 of SEQ ID NO:4.
16. A pharmaceutical composition comprising a protein of claim 11 and a pharmaceutically acceptable carrier.
17. A protein produced according to the process of claim 10.

18. A composition comprising an antibody which specifically reacts with a protein of claim 11.

19. A method of identifying an inhibitor of IL-13 binding to the IL-13 receptor which comprises:

- (a) combining a protein of claim 11 with IL-13 or a fragment thereof, said combination forming a first binding mixture;
- (b) measuring the amount of binding between the protein and the IL-13 or fragment in the first binding mixture;
- (c) combining a compound with the protein and the IL-13 or fragment to form a second binding mixture;
- (d) measuring the amount of binding in the second binding mixture; and
- (e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting IL-13 binding to the IL-13 receptor when a decrease in the amount of binding of the second binding mixture occurs.

20. An inhibitor identified by the method of claim 19.

21. A pharmaceutical composition comprising the inhibitor of claim 20 and a pharmaceutically acceptable carrier.

22. A method of inhibiting binding of IL-13 to the IL-13 receptor in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 21.

23. A method of inhibiting binding of IL-13 to the IL-13 receptor in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 16.

24. A method of inhibiting binding of IL-13 to the IL-13 receptor in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 18.

25. An isolated polynucleotide comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;
- (c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;
- (d) the amino acid sequence of SEQ ID NO:4;
- (e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;
- (f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and
- (g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain.

26. The protein of claim 11 wherein said amino acid sequence is part of a fusion protein.
27. The protein of claim 26 comprising an Fc fragment.
28. A method of treating an Ig-mediated condition in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 16.
29. The method of claim 28 wherein said condition is an IgE-mediated condition.
30. The method of claim 29 wherein said condition is selected from the group consisting of an allergic condition, asthma and an immune complex disease.
31. The method of claim 30 wherein said condition is selected from the group consisting of lupus, nephritis, thyroiditis and Grave's disease.
32. A method for potentiating IL-13 activity, said method comprising combining a protein having IL-13 activity with a protein of claim 11 and contacting such combination with a cell expressing at least one chain of IL-13R other than IL-13bc.

33. The method of claim 32 wherein the contacting step is performed by administering a therapeutically effective amount of such combination to a mammalian subject.

34. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331.

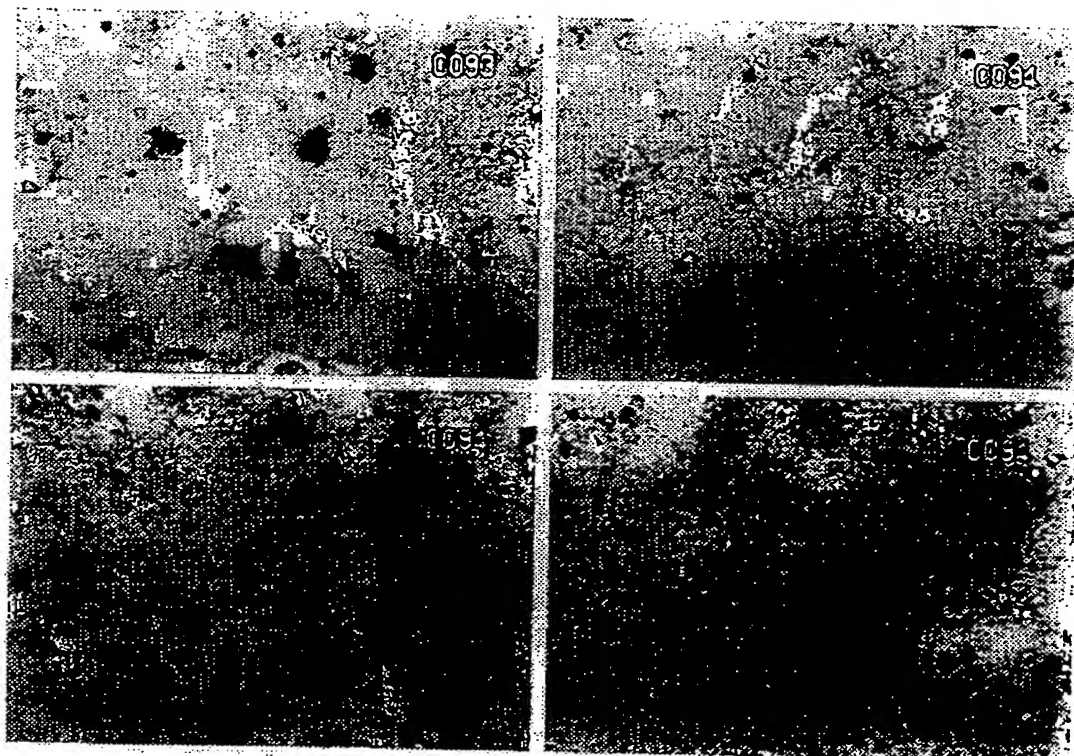
35. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

36. The polynucleotide of claim 25 encoding a peptide or protein comprising the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331

37. The polynucleotide of claim 25 encoding a peptide or protein comprising the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

IL-13

IL-11



IL-4

Mock

Fig. 1

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)